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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER
BERTOGLIO, VALERIE E

ART UNIT	PAPER NUMBER
1632	

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13

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 10/006,611	Applicant(s) NEZU ET AL.	
	Examiner Valarie Bertoglio	Art Unit 1632	

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on *15 October 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *1-15* is/are pending in the application.

4a) Of the above claim(s) *16-24* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1-15* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional applic

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *10* .
4) ☐ Interview Summary (PTO-413) Paper No(s).
5) ☐ Notice of Informal Patent Application (PTO-1
6) ☒ Other: *Detailed Action* .

DETAILED ACTION

Applicant's Amendment, filed October 15, 2002, paper No. 12, has been entered..

Election/Restrictions

Applicant's election of Group I, claims 1-15 in paper No. 12, without traverse is acknowledged. Claims 16-24 have been withdrawn from consideration. Claims 1-15 are under current examination

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-15 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claimed invention is directed to a transgenic, non-human mammal in which the suppression of expression of an endogenous LKB1 gene can be induced (claim 1) or is induced (claims 10). Claims 2 and 11 require suppression is induced by deletion of at least part of the LKB1 gene or a regulatory region thereof. Claim 3 requires part of the LKB1 gene be flanked by loxP sites. The claims encompass four genetically distinct animals: 1) a transgenic, non-human mammal with a transgene comprising 3 loxP sites and a neomycin resistance gene inserted in the endogenous LKB1 gene that disrupts the LKB1 gene (Figure 5, line 3), 2) a transgenic, non-human mammal made as a result

of Cre recombinase-mediated recombination of the transgene in mammal 1 wherein deletion of the neo gene results in allowing expression of the once disrupted LKB1 gene (Figure 7, line 5), 3) a transgenic, non-human mammal made as a result of Cre recombinase-mediated recombination of the transgene in mammal 1 wherein exons 2-8 of the LKB1 gene have been removed, resulting in a lack of LKB1 activity (Figure 7, line 4) and 4) a transgenic, non-human mammal wherein a functional loxP-flanked LKB1 transgene (mammal 3) has been disrupted in a postnatal animal by Cre recombinase-mediated deletion of a portion of the LKB1 gene (Figure 7, line 5).

The state of the art at the time of filing was such that one of skill could not predict the phenotype of transgenics. Leonard (1995, Immunological Reviews, Vol. 148, pages 98-113) disclosed mice with a disruption in the g_c gene which were intended to be a model for X-linked severe combined immunodeficiency (XSCID), but display a variety of unexpected traits (abstract). These knockout mice were expected to have thymocytes with decreased proliferation in response to stimulation with antibodies, but the thymocytes proliferated normally (page 105, line 7). Moens (1993, Development, Vol. 119, pages 485-499) taught two mutations produced by homologous recombination in two different locations of the N-myc gene produce two different phenotypes in mouse embryonic stem cells, one leaky and one null (page 486, column 1, first full paragraph). Griffiths (1998, Microscopy Research and Technique, Vol. 41, pages 344-358) teaches that, despite a known role for the PLP gene based on spontaneous mutations in the gene, the knockout mouse failed to display any of the expected phenotypes (page 350, last paragraph). Thus, the phenotype of knockout mice was unpredictable.

The species-specific requirements for transgene design are not clearly understood. Examples in the literature aptly demonstrate that even closely related species carrying the same transgene construct can exhibit widely varying phenotypes. For example, several animal models of human diseases have relied on transgenic rats when the development of mouse models was not feasible. Mullins (1990, Nature, Vol. 344, 541-544) produced outbred Sprague-Dawley x WKY rats with hypertension caused by expression of a mouse *Ren-2* renin transgene. Hammer (1990, Cell, Vol. 63, 1099-1112) describe spontaneous inflammatory disease in inbred Fischer and Lewis rats expressing human class I major histocompatibility allele HLA-B27 and human β_2-microglobulin transgenes. Both investigations were preceded by the failure to develop human disease-like symptoms in transgenic mice (Mullins, 1989, EMBO J., vol. 8, pages 4065-4072; Taurog, 1988, Jour. Immunol., Vol. 141, pages 4020-4023) expressing the same transgenes that successfully caused the desired symptoms in transgenic rats. Thus, the combination of elements (protein, promoter, species of protein, and species of transgenic) required to obtain a desired effect were not within the realm of routine experimentation at the time of filing.

Not only is the difference in transgenic mice and rats unpredictable for reasons stated above, the art at the time of filing was such that a number of significant limitations regarding the production of non-human transgenic animals existed. Wall (1996, Theriogenology, Vol. 45, pages 57-68) disclosed the unpredictability of transgene behavior due to factors such as position effect and unidentified control elements resulting in a lack of transgene expression or variable expression (paragraph bridging

pages 61-62). Overbeek (1994, "Factors affecting transgenic animal production," Transgenic animal technology, pages 96-98) taught that within one litter of transgenic mice, considerable variation in the level of transgene expression occurs between founder animals and causes different phenotypes (page 96, last paragraph). Therefore, it was unpredictable at the time of filing what gene of interest, promoter, enhancer, coding, or non-coding sequences present in the transgene construct, site of integration, method used and phenotype obtained were required to make a transgenic non-human mammal of interest.

The art at the time of filing further held that targeted gene insertion technology was not available for any species other than mouse. Since homologous recombination is required for gene targeting methods, embryonic stem cell technology must be available to carry out the method in most mammals. Mullins (1996, J. Clin. Invest., Vol. 98, pages S37-S40) teach that non-mouse ES cells capable of providing germline chimeras were not available (page S38, column 1, first paragraph). Campbell and Wilmut (1997, Theriogenology, vol. 47, pp, 63-72) acknowledge reports of ES-like cells in a number of species, but emphasize that as yet there are no reports of any cells lines that contribute to the germ line in any species other than mouse (page 65). Furthermore, the state of the art at the time of filing was that other potential methods of generating transgenic embryos using homologous recombination had not been developed (refer to McGreath, 2000, Nature, Vol. 405, pages 1066-1069; Kent-First, 2000, Nature Biotechnology, Vol. 18, pages 928-929; Dinnyes et al, 2002, Cloning and

Stem Cells, Vol. 4, pages 81-90). Thus, at the time of filing, knockout animals could not be prepared for any species other than mouse.

The specification teaches a transgenic mouse comprising a disruption in the LKB1 gene wherein the disruption is caused by a transgene comprising a 5' and 3' LKB-1homology arms, loxP-flanked neomycin resistance gene, and a 3' loxP site into the LKB1 gene (Figure 10, top) or by Cre-dependent excision of a loxP flanked recombinant LKB-1 gene (Figure 10, bottom).

1) The specification fails to enable using a transgenic, non-human mammal in which the suppression of expression of an endogenous LKB1 gene is induced or can be induced. The specification states that the claimed transgenic, non-human mammals can be used to study a variety of disease states (page 27, lines 8-11), however, the claims do not require that the transgenic animals have a phenotype that reflect a disease state. In fact, the specification teaches that the homozygous LKB1 mutant mice die in utero. The specification does not disclose a phenotype for heterozygotes that correlates to a disease state. The specification does not overcome the unpredictability in the art such that one of skill in the art would be able to determine the phenotype obtained. It would require one of skill in the art at the time the invention was made undue experimentation to determine when the desired phenotype has been reached and how to use the animal as a model of disease.

2) The specification fails to enable disrupting LKB1 function in a post-natal animal. The purpose of the invention is to generate a transgenic animal that expresses LKB1 during embryogenesis wherein LKB1 function can be subsequently removed by

disrupting the LKB1 gene postnatally. The specification discloses a means of performing this task by inserting non-disruptive loxP sites in the LKB1 gene. This is done through sequential disruptive transgene insertion into the LKB1 gene (Figure 7, line 3) followed by Cre-recombinase mediated removal of the disruptive neo-resistance gene sequences of the transgene (Figure 7, line 4). By removing the neo-resistance gene, animals comprising loxP sites flanking exons 2 and 8 are able to survive as phenotypically wild type. The invention teaches the use of Cre-recombinase in post-natal mice to remove exons 2-8, thus disrupting the LKB1 gene and potentially causing a disease state. The specification does not disclose how to introduce Cre recombinase to a postnatal animal or, if a transgene were used to introduce Cre, what promoter(s) should be used to express Cre. It is not clear when and where Cre expression is necessary to induce a loss of LKB1 gene function so as to cause disease state. It is further unclear what level of Cre expression is necessary to cause recombination of the LKB1 transgene in enough cells as to cause a disease state. Due to the unpredictability in the art of making transgenic animals, it would not be clear how to design a transgene such that Cre recombinase expression would result in homozygous disruption of the LKB1 gene and cause a phenotype. Furthermore, it would require one of skill in the art at the time the invention was made, undue experimentation to determine how to introduce or express Cre recombinase in an animal as to cause disruption of a loxP-flanked LKB1 gene.

3) The specification does not provide adequate guidance for one of skill in the art to generate non-human transgenic mammals in which the suppression of expression of

LKB1 can be induced, in any species other than mice. The guidance offered in the specification is limited to the production of knockout mice using gene targeting in mouse ES cells. The specification and the art at the time of filing fail to disclose any ES cells other than mouse ES cells. While the specification discloses the idea of generating transgenic mammals other than mice using homologous recombination in somatic cells followed by nuclear transfer of the transgenic nucleus into an oocyte, as stated above this technology had not been established in the art at the time of filing and was not described in the specification as to enable one skilled in the art at the time of the invention to carry out such a procedure. Without guidance as to how to generate ES cells that can populate the germline in species other than mouse or without guidance as to how to perform homologous recombination in somatic cells followed by nuclear transfer of the transgenic nucleus into an oocyte to generate a transgenic mammal, it would have required undue experimentation for one of skill in the art at the time the invention was made to make any transgenic, non-human mammal.

4) Applicants fail to enable making or using a transgenic, non-human mammal wherein the suppression of expression of an endogenous LKB1 gene is induced by deleting a regulatory region of the LKB1 gene (claims 2 and 11). The specification teaches inserting loxP sites into the recombinant LKB1 transgene such that expression of Cre recombinase results in deletion of exons 2 through 8 of the LKB1 gene. The specification does not teach any regulatory regions of the LKB1 gene or how to place loxP sites in the recombinant LKB1 transgene such that Cre recombinase-dependent excision results in the deletion of an LKB1 regulatory region.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-15 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The phrase "suppression... ...can be induced" (claim 1) is indefinite because it does not clearly set forth that suppression occurs. It is unclear whether the "suppression" occurs or is merely is capable of occurring. "Can" implies a latent property and the conditions for obtaining the latent property must be clearly defined. The claims do not clearly recite the conditions for obtaining such a property. Therefore, it is unclear if the latent property is ever obtained. The term "induced" in context of suppressing expression of LKB1 is indefinite. The metes and bounds of what applicants consider "induced" cannot be determined. It is unclear how a suppressed LKB1 gene that is "induced" differs from a suppressed LKB1 gene. Therefore, the term "induced" does not further limit the "suppression of expression." The claim must clearly set forth the structure of the genome of the mammal claimed stating that the LKB1 is disrupted. For example, "a transgenic, non-human mammal whose genome comprises a disruption in the LKB1 gene" would be clear. If the mammal has an LKB1 gene in which the disruption has not occurred, then the structure of the LKB1 gene that is capable of being disrupted must be clearly set forth by describing the loxP site in the LKB1 gene. As such it cannot be determined how "suppression" (claim 2), "inducibly suppressed" (claim 10) and "is suppressed by" (claim 11) correlate or further limit to claim 1. It is not clear

when or what the "induction step" is and if the suppression of expression is a result of insertion of the LKB1 transgene. It is further unclear whether suppression of the endogenous LKB1 gene through any means such as use of antisense RNA, protein inhibitors, targeted gene insertion, recombinase-mediated gene excision etc. is encompassed by the claim.

It is unclear if claim 2 is referring to deletion of the transgene that had been inserted into the endogenous LKB1 gene by homologous recombination or to direct deletion of the endogenous LKB1 gene.

The use of the phrases "expression of the endogenous LKB1 gene is inducibly suppressed" render claim 10 unclear. The term "inducible suppression" alludes to the use of Cre-recombinase to remove a region of the LKB1 gene that is flanked by loxP sites. However, this region is comprised of a recombinant transgene and Cre-mediated excision does not result in the suppression of expression of the endogenous gene. Expression of the endogenous gene is suppressed by the transgene insertion, not the Cre-mediated transgene deletion.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by Mullins (1989, The EMBO Journal, Vol. 8, pages 4065-4072).

Mullins taught a DBA/2J Ren-2 mouse in which the suppression of expression of an endogenous LKB1 gene can be induced. It was well known in the art at the time of filing, that suppression of expression of any endogenous gene, including LKB1, could be induced in a mouse using gene knockout technology. Thus, Mullins anticipates the claim.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

1) Claims 1,4,7,10-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Capecchi (*Scientific American*, 1994, vol. 270, pp 34-41) in view of Hemminki (1998, Nature, Vol. 391, pages 184-187).

Capecchi taught a mouse whose genome comprised a disruption in the HoxA-3 gene by insertion of a selective marker gene into the HoxA-3 gene. Capecchi differs from the claimed invention in that the targeting construct does not disrupt the LKB1 gene.

However, at the time the claimed invention was made, Hemminki taught the nucleic acid sequence of the LKB1 gene (page 185, paragraph bridging columns 1 and 2).

Accordingly, it would have been obvious for one of ordinary skill in the art at the time the claimed invention was made, to make a knockout mouse having a disruption in a targeted gene as taught by Capecchi wherein the gene was LKB1 as taught by Hemminki. One of ordinary skill in the art would have been sufficiently motivated to replace the Hox3A gene with the LKB1 gene, as it was an art-recognized goal to determine the physiological role of a gene of interest by the generation of a knockout mouse. One of ordinary skill in the art would have been sufficiently motivated to disrupt the LKB1 gene in mice to develop an animal disease model and determine the role of LKB1 in development as suggested by Hemminki (page 187, 1st paragraph). Inserting a transgene comprising a selective marker gene into the LKB1 gene is "inducing" the suppression of LKB1 expression as it is a process step that results in a lack of functional LKB1 gene expression.

Note that absent any phenotypic requirements for the claimed transgenic mouse, the combination of the cited prior art is sufficient to make obvious the claimed invention.

Thus, the claimed invention is clearly *prima facie* obvious in the absence of evidence to the contrary.

2) Claims 1-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Orban (*PNAS*, 1992, vol. 89, pp 6861-6865) in view of Hemminki (1998, Nature, Vol. 391, pages 184-187).

Orban taught a mouse whose genome comprises a β-galactosidase gene inserted between a pair of loxP sites (page 6861, column 1, 'Transgene Construction and Transgenic Mouse Production' and Figure 1B). In the presence of Cre-recombinase in thymocytes (page 6862, column 1, last paragraph) the β-galactosidase gene is excised and removed from the genome of the mouse thymocytes (page 6862, column 1, paragraph 1, lines 5-8 and last paragraph; Figure 1C). Orban differs from the claimed invention in that the gene between the loxP sites is not the LKB1 gene or regulatory region.

However, at the time the claimed invention was made, Hemminki taught sequence of the mouse LKB1 gene (page 185, paragraph bridging columns 1 and 2).

Accordingly, it would have been obvious for one of ordinary skill in the art at the time the claimed invention was made to make a mouse comprising a gene inserted between a pair of loxP sites as taught by Orban wherein the gene was LKB1 as taught by Ko. One of ordinary skill in the art would have been sufficiently motivated to replace the β-galactosidase gene with the LKB1 gene for the purpose of Cre-mediated excision of the gene after replacing the endogenous LKB1 gene with the transgene, as it was an art-recognized goal to determine the physiological role of a gene of interest by the generation of a knockout mouse and to control when excision occurred. One of ordinary skill in the art would have been sufficiently motivated to insert a region of LKB1 gene between a pair of loxP sites in mice for the purpose of generating a knockout of the LKB1 gene as there was motivation to develop an animal disease model and determine the role of LKB1 in development, as described by Hemminki (page 187, 1st paragraph).

Thus, the claimed invention is clearly *prima facie* obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Valarie Bertoglio whose telephone number is 703-305-5469. The examiner can normally be reached on 7:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Reynolds can be reached on 703-305-4051. The fax phone numbers for the organization where this application or proceeding is assigned are 703-872-9306 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-1234.

Valarie Bertoglio
Patent Examiner

MICHAEL C. WILSON
PATENT EXAMINER